



Publication number: **0 567 353 A1**

(12)

**EUROPEAN PATENT APPLICATION**

(21) Application number: **93303250.0**

(51) Int. Cl.<sup>5</sup>: **A61K 31/59**

(22) Date of filing: **26.04.93**

(30) Priority: **24.04.92 US 873389**

(43) Date of publication of application:  
**27.10.93 Bulletin 93/43**

(84) Designated Contracting States:  
**BE CH DE DK ES FR GB IE IT LI NL PT SE**

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(54) Use of 1-alpha,25-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> for the manufacture of a medicament for the treatment of osteoporosis.

(57) A method of treating osteoporosis comprising the administration of an effective amount of 1 $\alpha$ ,25-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub>.

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**Background of the Invention**

This invention relates to a method for treating or for preventing the depletion of calcium from bones as a result of osteoporosis.

5 More specifically, this invention relates to a method for treating or preventing various known forms of osteoporosis, e.g. postmenopausal, senile and steroid-induced osteoporosis, one of the characteristics of which is the loss of bone mass.

10 It is well known that females at the time of menopause suffer a marked loss of bone mass giving rise ultimately to osteopenia, which in turn gives rise to spontaneous crush fractures of the vertebrae and fractures of the long bones. This disease is generally known as postmenopausal osteoporosis and presents a major medical problem, both in the United States and most other countries where the life-span of females reaches ages of at least 60 and 70 years. Generally, the disease which is often accompanied by bone pain and decreased physical activity, is diagnosed by one or two vertebral crush fractures with evidence of diminished bone mass. It is known that this disease is accompanied by diminished ability to absorb calcium, decreased levels of sex hormones, especially estrogen and androgen, and a negative calcium balance.

15 Similar symptoms of bone loss characterize senile osteoporosis and steroid-induced osteoporosis, the latter being a recognized result of long term glucocorticoid (cortico-steroid) therapy for certain disease states.

Methods for treating the disease have varied considerably but to date no totally satisfactory treatment is yet known. A conventional treatment is to administer a calcium supplement to the patient. However, calcium supplementation by itself has not been successful in preventing or curing the disease. Another conventional treatment is the injection of sex hormones, especially estrogen, which has been reported to be effective in preventing the rapid loss of bone mass experienced in postmenopausal women. This technique, however, has been complicated by the fear of its possible carcinogenicity. Other treatments for which variable results have been reported, have included a combination of vitamin D in large doses, calcium and fluoride. The primary problem with this approach is that fluoride induces structurally unsound bone, called woven bone, and in addition, produces a number of side effects such as increased incidence of fractures and gastrointestinal reaction to the large amounts of fluoride administered. Another suggested method is to block bone resorption by injecting calcitonin or providing phosphonates.

20 U. S. Patent No. 4,225,596 suggests the use of various metabolites of vitamin D<sub>3</sub> for increasing calcium absorption and retention within the body of mammals displaying evidence of or having a physiological tendency toward loss of bone mass. The metabolites specifically named in that patent, i.e., 1 $\alpha$ -hydroxyvitamin D<sub>3</sub>, 1 $\alpha$ -hydroxyvitamin D<sub>2</sub>, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>2</sub> and 1,24,25-trihydroxyvitamin D<sub>3</sub>, although capable of the activity described and claimed in that patent, are also characterized by the disadvantage of causing hypercalcemia, especially if used with the conventional calcium supplement treatment. Therefore, use of these compounds to treat osteoporosis has not been widely accepted. U. S. Patent Nos. 3,833,622 and 3,901,928 respectively suggest using the hydrate of 25-hydroxyvitamin D<sub>3</sub> and 1 $\alpha$ -hydroxyvitamin D<sub>3</sub> for treatment of osteoporosis in a general expression of utility for those compounds. It is well known that both of those compounds express traditional vitamin D-like activity, including the danger of hypercalcemia.

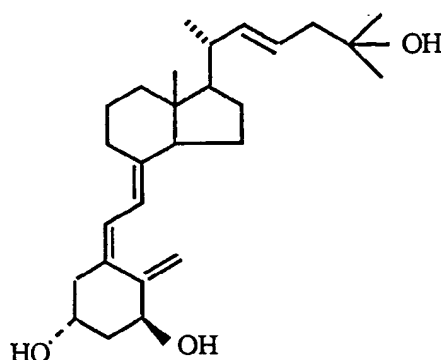
40 U. S. Patent No. 4,588,716 also suggests the use of 1 $\alpha$ ,25-dihydroxy-24-epi-vitamin D<sub>2</sub> to treat bone disorders characterized by the loss of bone mass, such as osteoporosis. Although this compound expresses some of the vitamin D-like characteristics affecting calcium metabolism such as increasing intestinal calcium transport and stimulating the mineralization of new bone, it has the advantage of minimal effectiveness in mobilizing calcium from bone. The 24-epi compound may be administered alone or in combination with a bone mobilization-inducing compound such as a hormone or vitamin D compound such as 1 $\alpha$ -hydroxyvitamin D<sub>3</sub> or -D<sub>2</sub>, or 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> or -D<sub>2</sub>.

45 It has now been found, according to the present invention, that the loss of bone mass, which is characteristic of osteoporosis may be effectively treated by the administration of a particular 1 $\alpha$ -hydroxylated vitamin D<sub>3</sub> compound in sufficient amounts to increase bone mass. According to the present invention there is provided 1 $\alpha$ ,25-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> or a hydroxyprotected derivative thereof for use in the production of a preparation for treating osteoporosis. The above compound (or a hydroxy protected derivative) may be administered alone or in combination with other pharmaceutically acceptable agents. Dosages of from not less than about 0.5 $\mu$ g/day to not more than about 3  $\mu$ g/day of the individual compound per se, or in combinations, are generally effective. This method has the distinct advantage that it will restore bone mass due to the insignificant bone mobilization activity of this compound and further this compound advantageously will not cause hypercalcemia even if the compound is administered continuously on a daily basis, as long as the appropriate compound dosages are used, it being understood that the dosage levels will be adjusted dependent on the response of the subject as monitored by methods known to those skilled in the art.

The above method, involving the administration of the indicated dosages of 1 $\alpha$ ,25-dihydroxy-22(E)-dehydro-

dro-vitamin D<sub>3</sub> compound is effective in restoring or maintaining bone mass, and thus provides a novel method for the treatment or prevention of various forms of osteoporosis such as postmenopausal osteoporosis, senile osteoporosis and steroid-induced osteoporosis. It will be evident that the method will find ready application for the prevention or treatment of disease states other than those named, in which the loss of bone mass is an indication.

The vitamin D compound useful in the present treatment is 1 $\alpha$ ,25-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> which can be prepared, for example by in vitro enzymatic hydroxylation at C-25 using a liver homogenate prepared from vitamin D-deficient rats (see, e.g. US-A-4307025) of the corresponding 1 $\alpha$ -hydroxy compound which can be prepared as described in journal of Bio. Org. Chem. 15, 152-166 (1987), Siciniski et al. "Δ<sup>22</sup>" unsaturated analogues of vitamin D<sub>2</sub> and their hydroxylated derivatives. The above compound may be administered alone or in combination with other pharmaceutically acceptable agents. 1 $\alpha$ ,24(R)-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> is characterised by the following structural formula:



A hydroxy protected derivative is one in which at least one of the hydroxy groups is replaced by a hydroxy protecting group.

The term "hydroxy-protecting group" refers to any group used for the protection of a hydroxy function during subsequent reaction including, for example, acyl or alkylsilyl groups such as trimethylsilyl, triethylsilyl, t-butyl-dimethylsilyl and analogous alkylated silyl radicals, or alkoxyalkyl groups such as methoxymethyl, ethoxymethyl, methoxyethoxymethyl, tetrahydrofuranyl or tetrahydropyranyl. A "protected-hydroxy" is a hydroxy function derivatized by one of the above hydroxy-protecting groupings.

As used herein, an acyl group is an alkanoyl group of 1 to 6 carbons in all its isomeric forms, or an aroyl group, such as benzoyl, or halo-, nitro- or alkyl - substituted benzoyl groups, or a dicarboxylic acyl group such as oxalyl, malonyl, succinoyl, glutaroyl, or adipoyl. "Alkyl" represents a straight-chain or branched saturated hydrocarbon radical of 1 to 5 carbons in all its isomeric forms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl or pentyl.

The vitamin D compound or combinations thereof can be readily administered as sterile parenteral solutions by injection or intravenously, or by alimentary canal in the form of oral dosages, or trans-dermally, or by suppository. Doses of from about 0.5 micrograms to about 3 micrograms per day of 1 $\alpha$ ,25-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> compound per se, or in combination with other 1 $\alpha$ -hydroxylated vitamin D compounds, the proportions of each of the compounds in the combination being dependent upon the particular disease state being addressed and the degree of bone mineralization and/or bone mobilization desired, are generally effective to practice the present invention. In all cases sufficient amounts of the compound should be used to restore bone mass. Amounts in excess of about 3 micrograms per day of 1 $\alpha$ ,25-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> or the combination of that compound with other 1 $\alpha$ -hydroxylated vitamin D compounds, are generally unnecessary to achieve the desired results, may result in hypercalcemia, and may not be an economically sound practice. In practice the higher doses are used where therapeutic treatment of a disease state is the desired end while the lower doses are generally used for prophylactic purposes, it being understood that the specific dosage administered in any given case will be adjusted in accordance with the specific compounds being administered, the disease to be treated, the condition of the subject and the other relevant medical facts that may modify the activity of the drug or the response of the subject, as is well known by those skilled in the art. For example, to be effective, 1 $\alpha$ ,25-dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> is preferably administered in a dosage range of 0.5-3  $\mu$ g/day. In general, either a single daily dose or divided daily dosages may be employed, as is well known in the art.

Dosage forms of the various compounds can be prepared by combining them with non-toxic pharmaceut-

ically acceptable carriers to make either immediate release or slow release formulations, as is well known in the art. Such carriers may be either solid or liquid such as, for example, corn starch, lactose, sucrose, peanut oil, olive oil, sesame oil and propylene glycol. If a solid carrier is used the dosage form of the compounds may be tablets, capsules, powders, troches or lozenges. If a liquid carrier is used, soft gelatin capsules, or syrup or liquid suspensions, emulsions or solutions may be the dosage form. The dosage forms may also contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, etc. They may also contain other therapeutically valuable substances.

The following Examples will serve to illustrate the efficacy of the present treatment method and its suitability for the prevention or treatment of osteoporosis.

#### EXAMPLE 1

This example illustrates that administration of  $1\alpha,25$ -dihydroxy-22(E)-dehydro-vitamin  $D_3$  at the recommended dosages will not mobilize calcium from bone whereas  $1\alpha,25$ -(OH) $_2D_3$  is strongly active in this regard. In animals on a low calcium diet, the elevation of serum calcium reflects the mobilization of calcium from bone.

In the case of the serum calcium data presented in Table I, weanling rats were fed a 0.02% calcium, 0.3% phosphorus vitamin D-deficient diet for three weeks. They were then implanted with Alzet minipumps that delivered the indicated dose of compounds in 95% propylene glycol, 5% ethanol each day for seven days. The rats were then killed and used for everted sac measurement of intestinal calcium transport and serum calcium measurements. The results are illustrated in Table I. The results show that  $1\alpha,25$ -dihydroxy-22(E)-dehydro-vitamin  $D_3$  is almost as active as  $1\alpha,25$ -(OH) $_2D_3$  in causing absorption of calcium from the small intestine. With respect to bone mobilization activity, a serum calcium of  $5.0 \pm 0.1$  indicates that  $\Delta^{22,22E}$ - $1\alpha,25$ -(OH) $_2D_3$  has some activity in mobilizing calcium from bone, but this activity is still much less than  $1\alpha,25$ -(OH) $_2D_3$  which has a serum calcium of  $5.7 \pm 0.1$ . As shown,  $1\alpha,25$ -(OH) $_2D_3$  is clearly very active in bone mobilization i.e. it raises serum calcium at the expense of bone.

**TABLE I**

INTESTINAL CALCIUM TRANSPORT AND MOBILIZATION OF CALCIUM FROM BONE IN RESPONSE TO THE VITAMIN D COMPOUNDS			
COMPOUND	DOSE	INTESTINAL CALCIUM TRANSPORT SEROSAL CALCIUM/MUCOSAL CALCIUM	SERUM CALCIUM mg %
None	0	$3.0 \pm 0.2$	$4.2 \pm 0.2$
$1\alpha,25$ -(OH) $_2D_3$	130	$8.5 \pm 0.9$	$5.7 \pm 0.1$
$\Delta^{22,22E}$ - $1\alpha,25$ -(OH) $_2D_3$	130	$7.7 \pm 0.6$	$5.0 \pm 0.1$

#### EXAMPLE 2

This example illustrates the anti-rachitic activity of  $1\alpha,25$ -dihydroxy-22(E)-dehydro-vitamin  $D_3$  in male rats. In this example, weanling male rats were fed a 1.2% calcium, 0.1% phosphorus, vitamin D-deficient diet for three weeks. The indicated dosages of test compounds or  $1\alpha,25$ -(OH) $_2D_2$  were injected intraperitoneally in 0.1 ml 5% ethanol/95% propylene glycol daily for 7 days. The rats were killed and their femurs removed for bone ash determinations. There were eight rats per group and the values represent the average  $\pm$  S.E.M. The results are illustrated in Table II. The results show that animals treated with  $1\alpha,25$ -dihydroxy-22(E)-dehydro-vitamin  $D_3$  effectively increased bone mass over a control group, and that  $1\alpha,25$ -dihydroxy-22(E)-dehydro-vitamin  $D_3$  is similar to  $1\alpha,25$ -(OH) $_2D_3$  in its ability to stimulate new bone formation. This experiment coupled with Example 1 illustrates the extraordinary ability of this compound to elevate bone mass.

TABLE II

ANTI-RACHITIC ACTIVITY OF $\Delta^{22,22E},1\alpha,25$ -DIHYDROXYVITAMIN D <sub>3</sub>			
GROUP	AMT OF COMPOUND (pmol/day/7days)	TOTAL Mg Ash	% Ash
-D	0	41 ± 3	24.2 ± 2
1 $\alpha,25$ -(OH) <sub>2</sub> D <sub>3</sub>	65	55 ± 2	27 ± 1
	195	57 ± 8	29 ± 2
$\Delta^{22,22E}-1\alpha,25$ -(OH) <sub>2</sub> D <sub>3</sub>	65	53 ± 8	28 ± 2
	195	45 ± 6	26 ± 1

## EXAMPLE 3

This example illustrates that the compound of the present invention is effective for restoring bone mass in rats made osteoporotic by ovariectomy. Mature female rats were obtained from the Sprague Dawley Company of Madison, Wisconsin either as sham-operated or as oophorectomized rats. The animals were placed on a 0.25% calcium, 0.3% phosphorus, purified diet for three weeks at which time 8 sham-operated and 8 oophorectomized rats were sacrificed. At this time, both groups had 400 mg of ash per femur and the percent ash was 61 ± 2%, indicating fully mineralized skeleton or a lack of any evidence of osteomalacia. The remaining rats were then divided into groups of 8 according to the Table below. They were allowed to proceed on the diet for approximately 5 months in order to reduce mineral content of the femur. At this time, the animals were divided into the appropriate groups and dosed for 18 days with the compounds listed. The dosing was carried out in 95% propylene glycol/5% ethanol solution in an Alzet minipump that delivered 13  $\mu$ L solution per day and provided the indicated dose in pmol. At the end of the 18-day period, the animals were killed, the femurs removed, extracted with ethanol for 24 hours and with chloroform for 24 hours using a Soxhlet extractor and then dried to constant weight. They were then subjected to muffle furnace temperatures of 600°C for 24 hours. The remaining ash was dried to constant weight and recorded. The values represent total ash per femur or percent ash. There were eight animals per group and the results are expressed as the mean ± S.E.M. The results are illustrated in Table III. A comparison between the sham operated and oophorectomized animals reveals the bone lost as a result of estrogen lack. The increase in bone mass above the oophorectomized control in the dosed animals reveals the degree of bone restoration. It is clear from the data in Table III that  $\Delta^{22,22E}-1\alpha,25$ -(OH)<sub>2</sub>-D<sub>3</sub> is about as effective as 1 $\alpha,25$ -(OH)<sub>2</sub>-D<sub>3</sub> in restoring bone mass at the 65 pmol/day dosage as shown by the mg femur ash i.e. 383 ± 3.5mg versus 388 ± 7.1 mg. As a result,  $\Delta^{22,22E}-1\alpha,25$ -(OH)<sub>2</sub>-D<sub>3</sub> is effective for restoring bone mass.

TABLE III

THE RESTORATION OF BONE BY 1 $\alpha,25$ -(OH) <sub>2</sub> D <sub>3</sub> , AND $\Delta^{22,22E}-1\alpha,25$ -(OH) <sub>2</sub> -D <sub>3</sub> IN OOPHORECTOMIZED ADULT FEMALE RATS				
GROUP	DOSE (pmol/day)	BODY WEIGHT	FEMUR ASH	
			%	mg
Sham-operated	0	282 ± 6	62 ± 0.3	411 ± 7.8
Oophorectomized (OOX)	0	354 ± 11	59 ± 1.6	350 ± 2.4
OOX + 1 $\alpha,25$ -OH-D <sub>3</sub>	65	372 ± 7.0	59 ± 0.5	388 ± 7.1
OOX + $\Delta^{22,22E}$	65	365 ± 33	59 ± 0.3	383 ± 3.5
1 $\alpha,25$ -(OH) <sub>2</sub> -D <sub>3</sub>	195	371 ± 33	60 ± 0.1	416 ± 15

# Claims

- 5 1.  $1\alpha,25$ -Dihydroxy-22(E)-dehydro-vitamin D<sub>3</sub> or a hydroxy-protected derivative thereof for use in the production of a preparation for treating osteoporosis.
2. Use according to claim 1 wherein the osteoporosis is postmenopausal osteoporosis.
3. Use according to claim 2 wherein the vitamin D<sub>3</sub> compound is administered to women during and subsequent to menopause.
- 10 4. Use according to claim 2 wherein the vitamin D<sub>3</sub> compound is administered to women prior to the onset of menopause.
5. Use according to claim 1 wherein the osteoporosis is senile osteoporosis.
- 15 6. Use according to claim 1 wherein the osteoporosis is steroid-induced osteoporosis.
7. Use according to any one of claims 1 to 6 wherein the vitamin D<sub>3</sub> compound is administered in an amount from 0.5 to 3 micrograms per day.
- 20 8. Use according to any one of claims 1 to 7 wherein the vitamin D<sub>3</sub> compound, in solution in a liquid vehicle ingestible by and nontoxic to said patient, is administered orally in encapsulated form.
9. Use according to any one of claims 1 to 7 wherein the vitamin D<sub>3</sub> compound is administered in a slow release formulation.
- 25 10. Use according to any one of claims 1 to 7 wherein the vitamin D<sub>3</sub> compound is administered daily in divided dosages.

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Office

# EUROPEAN SEARCH REPORT

Application Number

EP 93 30 3250

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X Y	US-A-4 719 204 (HECTOR F. DELUCA ET AL.) * column 1, line 65 - column 2, line 20 * * column 10, line 44 - column 12, line 7 * ---	1,7,8 2-6,9,10	A61K31/59
Y	US-A-4 689 180 (HECTOR F. DELUCA ET AL.) * column 1, line 67 - column 2, line 31 * * column 4, line 20 - line 46 * ---	2-6,9,10	
X	J. NUTR. BIOCHEM. vol. 4, no. 1, January 1993, pages 49 - 57 TAI C. CHEN ET AL. 'An evaluation of 1,25-dihydroxyvitamin D3 analogues on the proliferation and differentiation of cultured human keratinocytes, calcium metabolism and the differentiation of human HL-60 cells' * abstract; figure 1 * * page 51, right column, paragraph 2 - page 52, left column, paragraph 1 * ---	1  2-10	
X	ARCH. TOXICOL. vol. 63, no. 5, 1989, pages 394 - 400 ANDREAS KISTLER ET AL. 'Effects of vitamin D derivatives on soft tissue calcification in neonatal and calcium mobilisation in adult rats' * tables 1,3 * -----	1  2-10	TECHNICAL FIELDS SEARCHED (Int. Cl.5)  A61K
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 23 JULY 1993	Examiner TZSCHOPPE D. A.
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document</p>			

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